Claims

1. An improved BRET assay comprising
i) adding a substrate to a cell comprising GPCR-Rluc fusion protein and a β-arrestin-GFP fusion protein, wherein the β arrestin is mutated,
ii) adding a ligand to obtain, if possible, a GPCR-Rluc/β-arrestin-GFP complex, and
iii) measuring a BRET signal to obtain a BRET ratio,

wherein the improvement leads to an increased BRET ratio compared with the ratios obtained by use of the same process employing a β-arrestin-GFP fusion protein wherein the β-arrestin is the wild type β-arrestin, or employing a β-arrestin-GFP fusion protein, wherein the β-arrestin is a β-arrestin specifically mutated so that it acts on the receptor independent of the receptors phosphorylation state.

2. An improved assay according to claim 1, wherein separation of β-arrestin-GFP from GPCR-Rluc/β-arrestin-GFP complex is delayed and/or inhibited.

3. An improved assay according to claim 1 or 2, wherein internalization of the GPCR-Rluc/β-arrestin-GFP complex is inhibited.

4. An improved assay according to any of the preceding claims, wherein β-arrestin is mutated so that its binding to clathrin and/or AP2 is impaired.

5. An improved assay according to any of the preceding claims, wherein β-arrestin is truncated so that it does not contain any clathrin and/or AP2 binding sites.

6. An improved assay according to any of the preceding claims, wherein β-arrestin is mutated by deletion, insertion or substitution so that one or more AP2 binding sites are impaired in their binding to AP2.

7. An improved assay according to any of the preceding claims, wherein β-arrestin is mutated so that its binding to phosphoinositide is impaired.

8. An improved assay according to any of the preceding claims, wherein the cell comprises a further amount of G-protein coupled receptor kinase (GRK) as compared to the amount of GRK naturally present in the cell.

9. An improved assay according to claim 8, wherein the G-protein coupled receptor kinase is GRK 2.

10. An improved assay according to claim 8, wherein the G-protein coupled receptor kinase is GRK 5.

11. An improved assay according to any of the preceding claims, wherein β-arrestin is further mutated so that it is phosphorylation independent.

12. An improved assay according to any of the preceding claims wherein β-arrestin is originating from an animal source, such as, e.g, from rodents, swine, poultry, cattle, sheep, goats, horses, cats, dogs, monkeys and humans.

13. An improved assay according to any of the preceding claims, wherein β-arrestin is a β-arrestin-1 or β-arrestin-2.

14. An improved assay according to any of the preceding claims, wherein the β-arrestin is a human β-arrestin-1 374 stop mutant or human β-arrestin-2 373 stop mutant.

15. An improved assay according to any of claims 1-13, wherein the β-arrestin is a human β-arrestin-2 R393E;R395E mutant.

16. An improved assay according to any of claims 1-13, wherein the β-arrestin is a human β-arrestin-2 R393A;R395A mutant.

17. An improved assay according to any of the preceding claims, wherein the β-arrestin is human β-arrestin-2 K233Q;R237Q;K251Q mutant.

18. An improved assay according to any of the preceding claims for use in drug discovery methods.

19. An improved assay according to any of claims 1-17 for use in high-throughput screening.

20. An improved assay according to any of the preceding claims, wherein the substrate is DeepBlueC™.

21. An improved assay according to any of the preceding claims, wherein the substrate is used in the form of a solution from which no visual precipitate is formed after storage at room temperature for at least 30 min such as, e.g., for at least about 45 min, for at least about 1 hr, for at least about 1.5 hrs, for at least about 2 hrs, for at least about 2.5 hrs, for at least about 3 hrs, for at least about 3.5 hrs or for at least about 4 hrs.

22. An improved assay according to claim 21, wherein the solution containing the substrate comprises one or more organic solvents.

23. An improved assay according to claim 22, wherein the one or more organic solvents are selected from alkanols including ethanol, propanol, isopropanol, and butanol.

24. An improved assay according to any of claims 20-23, wherein the solvent is EtOH.

25. An improved assay according to claim 24, wherein the solution comprises from about 15% v/v EtOH to about 100 v/v% EtOH, such as, e.g. from about 20% v/v EtOH to about 90% v/v EtOH, from about 30% v/v EtOH to about 80% v/v EtOH, from about 35% v/v EtOH to about 75% v/v EtOH, from about 35% v/v EtOH to about 70% v/v EtOH, from about 35% v/v EtOH to about 65% v/v EtOH, from about 35% v/v EtOH to about 60% v/v EtOH, from about 40% v/v EtOH to about 60% v/v EtOH, from about 40% v/v EtOH to about 55% v/v EtOH or from about 40% v/v EtOH to about 50% v/v EtOH.

26. An improved assay according to claim 25, wherein the solution comprises DeepBlueC™ in 40% v/v EtOH.

27. A solution comprising DeepBlueC™ and one or more organic solvents, wherein no visual precipitate is formed after storage at room temperature for at least 30 min such as, e.g., at least about 45 min, for at least about 1 hr, for at least about 1.5 hrs, for at least about 2 hrs, for at least about 2.5 hrs, for at least about 3 hrs, for at least about 3.5 hrs or for at least about 4 hrs.

28. A solution according to claim 27, wherein the one or more organic solvents are selected from alkanols including ethanol, propanol, isopropanol, and butanol.

29. A solution according to claim 28, wherein the solvent is EtOH.

30. A solution according to claim 29, comprising from about 15% v/v EtOH to about 100 v/v% EtOH, such as, e.g. from about 20% v/v EtOH to about 90% v/v EtOH, from about 30% v/v EtOH to about 80% v/v EtOH, from about 35% v/v EtOH to about 75% v/v EtOH, from about 35% v/v EtOH to about 70% v/v EtOH, from about 35% v/v EtOH to about 65% v/v EtOH, from about 35% v/v EtOH to about 60% v/v EtOH, from about 40% v/v EtOH to about 60% v/v EtOH, from about 40% v/v EtOH to about 55% v/v EtOH or from about 40% v/v EtOH to about 50% v/v EtOH.

31. A solution according to claim 30, comprising DeepBlueC™ in 40% v/v EtOH.

32. A method for preparing a solution according to any of claims 27-31, the method comprising diluting a stock solution of DeepBlueC™ in a solution comprising one or more organic solvents.

33. Use of an improved BRET assay according to any of claims 1-26 for identifying a GPCR ligand.

34. Use of an improved BRET assay according to claim 33, wherein the ligand is an agonist.

35. Use of an improved BRET assay according to claim 34, wherein the ligand is an antagonist.